DOI:
10.1016/j.ccell.2016.07.014

Document Version
Peer reviewed version

Combinatorial tethering: A novel mode to recruit non-canonical PRC1 for normal and malignant GC B cell development

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Summary

Polycomb repressive complexes (PRCs) are key to normal development and frequently deregulated in human cancer. In this issue of Cancer Cell, Beguelin et al. report a mechanism of non-canonical PRC1 recruitment by BCL6 in collaboration with EZH2 mediated H3K27me3 for establishment of stable repressive complexes in germinal center B cells.

During humoral response, while stimulated mature B cells will differentiate into antibody-secreting plasma cells, a subset transiently becomes germinal center (GC) B cells undergoing affinity maturation before differentiating into memory B cells or plasma cells. These GC B cells are also believed to be a major source of the most common B cell lymphomas (Nutt et al., 2015). At the molecular level, suppression of plasma cell transcriptional programs and cell cycle checkpoints (e.g., CDKN1A) is mediated by the H3K27me3 catalytic subunit of PRC2, EZH2, which is required for GC B cell development (Beguelin et al., 2013). The transcription factor Bcl6 is also required for GC B cell formation (Hatzi and Melnick, 2014). In line with their frequent deregulation in B cell lymphomas, mouse studies demonstrated that overexpression of Bcl6 (Cattoretti et al., 2005) or gain-of-function Ezh2 mutant (Beguelin et al., 2013) induced GC hyperplasia and sporadic GC lymphomas with extended latency, indicating their critical roles in B cell lymphoma. Mechanistically, it remains largely unclear how PRCs are recruited to the promoters and mediate critical repression program especially in the absence of key canonical PRC1 components such as PCGF2/MEL18 and PCGF4/BMI1 (Figure 1A).

Using various conditional mouse models, Beguelin et al. in this issue of Cancer Cell showed a reciprocal requirement between EZH2 and BCL6 for GC hyperplasia driven by BCL6 or gain-of-function $EZH2^{Y641F}$ mutant, respectively (Bejuelin et al., 2016). Furthermore, ChIP-seq and RNA-seq comparing naive B cells with GC B cells identified a significant subset (30%) of de novo H3K27me3/H3K4me3 bivalent and transcriptionally repressed genes in GC B cells bound by BCL6 and its interacting partner BCOR. Although EZH2 does not interact with BCL6, it is still recruited by unknown mechanisms to a subset of H3K4me3 marked promoters bound by BCL6 and BCOR. Targeting EZH2 or BCL6 alone (by chemical inhibitors or siRNA) led to de-repression of de novo bivalent genes bound by BCL6/BCOR, indicating their collaborative functions in maintaining gene repression. Interestingly, while several canonical PRC1 components were down-regulated in GC B cells, non-canonical PRC1 genes such as BCOR, PCGF1 and KDM2B were up-regulated. Consistently, genetic ablation of BCOR phenocopied loss of EZH2 or BCL6 and significantly reduced the number of GC B cells in $Ezh2(Y641F)^{fl/WT}$ transgenic mice. GC B cells required BCOR/BCL6 interaction, as shown using $Bcl6^{BTBmut}$ mice, which express a BCOR-binding deficient BCL6 mutant, or the BCOR/BCL6 interaction inhibitor FX1.

To delineate the molecular mechanisms underlying the recruitment of these various components for target gene repression, the authors further demonstrated that the significant reduction in PRC2 and BCOR occupancy and loss of H3K27me3 and H2A119ub marks by Ezh2 inhibitor treatment did not alter BCL6 binding. On the other hand, reciprocal experiments using FX1 led to a reduction in non-canonical PRC1/BCOR and BCL6 recruitment and H3K27me3 and H2A119ub marks, but did not change PRC2 occupancy, suggesting that BCL6 and EZH2 mediate tethering of non-canonical PRC1 to their targets (Figure 1B). Furthermore, chromodomain containing protein

CBX8 was identified as a part of the non-canonical PRC1 that was recruited to de novo bivalent promoters (Figure 1B). EZH2 inhibitor treatment reduced the levels of CBX8 binding, whereas shRNA mediated suppression of CBX8 decreased BCOR recruitment and H2A119ub resulting in de-repression of GC B cell bivalent genes. Genetic ablation of CBX8 in a mouse model led to significant depletion of GC B cells, consistently suggesting that CBX8 mediates the suppressive effects of non-canonical PRC1 complex and PRC2 by binding H3K27me3 marked chromatin. Together, their findings reveal a close cooperation between BCL6/non-canonical PRC1 and EZH2 where combinatorial tethering of non-canonical PRC1 by EZH2 and BCL6 is critical for GC B cell development, as either one alone is not sufficient for stable non-canonical PRC1 recruitment and function (Figure 1C).

Finally, to address if EZH2 and BCL6 cooperate in lymphoma development, the authors used an *Ezh2(Y641F)*$^{fl/WT}$;Cγ1-cre;IμBcl6 mouse model and revealed that both alleles did collaborate to induce lymphoma in vivo. Similar results were also observed using a bone marrow transplantation model (IμBcl6 bone marrow with/without EZH2^{Y641F} viral transduction). Importantly, while the use of EZH2 or BCL6 inhibitors alone showed anti-lymphoma activities in human lymphoma cells (Cerchietti et al., 2010; McCabe et al., 2012), combined targeting of EZH2 and BCL6/BCOR resulted in an even bigger de-repression of target genes and enhanced anti-lymphoma activity in xenograft models (Figure 1D), providing a strong rationale for combination therapy in this type of B cell lymphoma.

Recruitment of PRCs to their downstream targets in mammalian cells has been a long-standing question (Lau and So, 2015). This report proposes a collaborative combinatorial tethering mechanism mediated by EZH2/BCOR/BCL6 during GC B cell development, adding another level of complexity to the growing modes of PRC targeting (Klose et al., 2013). Therefore, although transcription factor BCL6 may recruit BCOR/PRC1 to their genomic targets in an instructive manner, a stable complex formation seems to require continuous PRC1 sampling of specific marks (such as H3K27me3 and CpG islands recognized by CBX8 and KDM2B, respectively) to sustain optimal epigenetic silencing mediated by both PRCs. While it remains unclear in this study how PRC2/EZH2 is recruited to this subset of H3K4me3 marked genes bound by BCL6 and BCOR, it is tempting to speculate that PRC2 may also be tethered to those genes in a combinatorial fashion by yet unidentified factors. On the other hand, almost 6 times more promoter regions are monovalently methylated (H3K27me3) by PRC2/EZH2 compared to bivalently methylated (H3K4me3 and H3K27me3). Only 3% of monovalent promoters are bound by BCL6/BCOR, whereas ~30% of de novo bivalent promoters are enriched in BCL6/BCOR. While this may suggest an important role of BCL6/BCOR in establishing bivalent genes critical for rapidly proliferating GC B cells and differentiation into antibody secreting plasma cells once the GC reaction is completed, there are still a significant proportion of monovalent genes specifically methylated by PRC2, which can also have

important functions in GC B cell development and hyperplasia. On the other hand, this bimodal epigenetic gene regulation in GC B cells may also act as a safeguard against lymphoma transformation, which requires additional and more stable epigenetic alternations.

Finally, although the cooperative effect between EZH2 mutant and BCL6 resulting in GC hyperplasia was already visible 10 days after immunization, it often took over 200 days with continuous immunizations for lymphoma onset, indicating that the transition from GC hyperplasia to lymphoma is relatively rare, even when both EZH2 and BCL6 are deregulated. One explanation for this is that GC B cells can still quite efficiently exit this state despite the presence of gain-of-function EZH2 mutation and concomitant H3K27 trimethylation. While it is generally believed that the exit of GC B cells follows downregulation of EZH2 (De Silva and Klein, 2015), the relatively unstable epigenetic silencing suggested by the combinatorial tethering model may in part explain the de-repression of target genes despite the presence of aberrant H3K27me3. On the other hand, the long disease latency indicates the need for additional mutations in EZH2/BCL6 deregulated cells for full-blown transformation. Nevertheless, the results from combined therapy indicate that the lymphomas are still largely dependent on functional EZH2 and BCL6, suggesting interference with combinatorial tethering of PRCs as a promising therapeutic intervention in this type of B cell lymphoma.

Acknowledgements

C.W.E.S is supported by Cancer Research UK (CRUK) and Bloodwise.

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